

demonstrated that holding times of as long as 48 hours show no appreciable change in bacterial levels contained in a sample. Thus, the use of longer hold times is indeed generally accepted in the field.

- To the extent a longer hold time has an effect on bacterial levels, the effect is that bacterial concentrations will be *lower* than if the analysis were done immediately.
- The methodology used for analyzing the CDM samples is thus generally accepted and based on good grounds, and the data from these analyses are useful and scientifically valid for assessing the extent of microbial contamination in IRW waters. The data collected do not exaggerate microbial contamination and associated human health risks in the IRW. If anything, they are an underestimate due to the tendency of indicator bacteria to die off with increased holding times.

In short, because the analyses of those CDM samples are reliable, expert opinions based upon the analyses of these samples are admissible under *Daubert*.

I. Background Facts

The purpose of analyzing the IRW water samples was to add to existing data collected by the State of Oklahoma and the USGS regarding the extent of bacterial contamination in the waters of the IRW and the percentage of samples that exceeded State and federal water quality guidelines. *See* Ex. 1 (Harwood Aff., ¶ 4).

At the start of the sampling program in 2005, procedures and laboratories were selected so that samples could arrive at the laboratory as soon as possible. *See* Ex. 2 (Olsen Aff., ¶ 6). Initially a laboratory close to the IRW was selected so that samples could arrive at the laboratory the same day of collection. *See id.* However, the quality of the bacterial analyses was determined not to be acceptable, so another laboratory was interviewed and selected. *See id.*

This laboratory was Environmental Microbiological Laboratories ("EML"), located in California. *See id.* The bacteria hold time issue was discussed and the decision was made that overnight shipping with analysis set-up the same day the sample was received at the laboratory was acceptable. *See id.*

The field staff who were collecting the samples in the IRW were consistently instructed to make sure the samples arrived at the laboratory as soon as possible. *See* Ex. 2 (Olsen Aff., ¶ 5). Contrary to Defendants' suggestions, there was *never* a "96 hour hold time" established for samples collected in the IRW, *see* Ex. 2 (Olsen Aff., ¶ 12),¹ and the field staff were never told that there was a 96-hour hold time for bacteria samples. *See* Ex. 2 (Olsen Aff., ¶ 5). In fact, just the opposite was true. The field staff made every attempt to make sure the samples arrived at EML as soon as possible. *See id.* In fact, CDM standard operating procedure (SOP 9-1, Shipping and Chain-of-Custody) states that "samples for bacteria analyses will be shipped overnight on the day they are collected," and this SOP was consistently followed. *See id.*

Additionally, samples were always packed in ice in coolers to maintain cold conditions consistent with recommended guidelines and CDM Standard Operating Procedure (SOP) No. 9.1 (section 2.4.3). *See* Ex. 2 (Olsen Aff., ¶ 9). The field staff were carefully instructed and experienced in the amounts of ice necessary to keep the samples at the recommended

¹ Defendants attempt to make hay of the fact that Dr. Olsen admittedly misremembered some of the facts surrounding the bacteria sampling protocol and supporting literature during his September 2008 deposition. *See* Motion, pp. 10-11. For example, he could not remember the specific references he had reviewed concerning bacterial holding times. *See* Ex. 2 (Olsen Aff., ¶ 10). However, as he explained in his deposition, Dr. Harwood made the final decision on bacterial hold times. *See id.*; *see also* Ex. 2 (Olsen Depo., pp. 148-49 & 153). Since his deposition, he located his working file containing the literature references discussed during his deposition. *See* Ex. 2 (Olsen Aff., ¶ 10). This file (which was produced to Defendants with Dr. Olsen's considered materials) contained, *inter alia*, Pope, et al., "Assessment of the effects of holding time and temperature on *Escherichia coli* densities in surface water samples," *Appl. Environ. Microbiol.*, 69:6201-07 (2003). *See id.*

temperature ($<8^{\circ}\text{C}$) before arrival at EML. *See id.* The samples were always received by EML in an acceptable state with appropriate amounts of ice. *See* Ex. 3 (Sambasivam Aff., ¶ 3). In addition, EML always started and performed the set-up for the bacterial analyses (cultures) on the day the samples were received. *See id.*

Because microorganisms are very small, and analyses done with microscopes are very labor-intensive, the general strategy in analysis of pathogens and indicator bacteria is to allow the organisms to grow for a period of time -- usually 24-48 hours -- so that a visual check of their growth is possible. *See* Ex. 1 (Harwood Aff., ¶ 5). This is called culture-based or culture-dependent analysis, and has been the standard for a century.² *See id.* Many standard methods for pathogen or indicator analysis require several culture-based steps that each require 1-2 days to perform. *See id.* Therefore the total analytical time required to confirm results can stretch out for over two weeks. *See id.* It is important to understand that the hold time or set-up time -- from sample collection to inoculation of the sample in the first culture medium -- is the crucial time period for ensuring that one does not underestimate the concentration of target microorganisms in the sample. *See id.*

Based largely on a study conducted in 1953 by the Public Health Laboratory Service Water Sub-Committee, regulatory agencies generally stipulate a maximum 6-hour hold time for microbiological analysis of surface water samples. *See* Ex. 1 (Harwood Aff., ¶ 6). This recommendation is stipulated for samples that are taken for regulatory compliance purposes -- e.g., beach water quality monitoring or assessment of ambient water quality for TMDL programs -- because bacteria tend to die off in samples that are held for long periods. *See id.* Importantly,

² In the last two decades culture-dependent methods have begun being augmented or supplemented by PCR-based, culture-independent methods. *See* Ex. 1 (Harwood Aff., ¶ 5).

the effect of extending the hold time is that bacterial concentrations will be lower than if the analysis was done immediately. *See id.*

Peer reviewed studies, however, have found either no significant differences from the 6-hour holding time results when samples are held 24-48 hours at 8-10° C (refrigerated or on ice), or decreases in bacterial concentrations. *See* Ex. 1 (Harwood Aff., ¶ 6). For example, the Public Health Laboratory Service Water Sub-Committee study on which EPA, USGS and ODEQ hold-time standards are based found that 21.5% of samples that were tested for fecal coliforms after 24 hours of refrigeration decreased in concentration, while only 3.5% of samples showed an increased concentration. *See id.* The majority of samples (75%), however, showed no change. *See id.* Because fewer samples showed a change when held for 6 hours compared to 24 hours (the only two times tested), the authors recommended the 6-hour hold time. *See id.*

Other studies have corroborated these findings. For instance, two studies, one published in 2003 and one published in 2004, have found that water samples held at refrigerator temperatures for 24 or up to 48 hours experience either no change or a decrease in bacterial concentrations. *See* Ex. 1 (Harwood Aff., ¶ 7). Further, the American Public Health Association's *Standard Methods for the Examination of Water and Wastewater* (2005) also stipulates that ambient water samples collected for non-regulatory purposes can be held for 24 hours at cold temperatures before analysis. *See id.* Yet further, a 1977 study presented a detailed literature review and an experimental holding time study for fecal coliforms. *See* Ex. 1 (Harwood Aff., ¶ 8). It found that the 24-hour holding time for samples analyzed for fecal coliforms produced equivalent results to a 4-hour hold time.³ *See id.*

³ Longer holding times were not analyzed in that study. *See* Ex. 1 (Harwood Aff., ¶ 8).

Dr. Harwood has also overseen hold-time studies performed in her laboratory (outside the litigation context), which corroborate these results. *See* Ex. 1 (Harwood Aff., ¶ 8). For example, fresh water samples held for approximately 24 hours on ice or in the refrigerator generally have unchanged bacterial concentrations compared to their counterparts held 6 hours or less. *See id.* Consistent with the studies above,⁴ Dr. Harwood found that, "if anything, bacterial concentrations decreased with holding time. Enterococci concentrations did not change significantly with a 48 hour hold time." *See id.*

The actual times -- from collection in the field to analysis set-up -- at EML are reflected in the following table:

Number of Samples for Each Sample Type⁵

Time (hr)	CDM River	USGS	Tenkiller	Residential Wells	Springs	EOF	HFS
<24	84	117	12	20	11	3	11
24 – 30	61	127	14	44	24	11	34
30 – 48	16	6	7	4	6	14	19
>48	16	1	7	5	0	39	61
Ave hr for >48	75 hr	69 hr	52 hr	88 hr	--	84 hr	195 hr

⁴ Among the studies cited by Dr. Harwood are Standridge, et al., *Comparison of Four-Hour and Twenty-Four-Hour Refrigerated Storage of Nonpotable Water for Fecal Coliform Analysis*, Appl. Environ. Microbiol., 34:398-402 (1977), attached as Exhibit 4; Selvakumar, et al., *Effects of Sample Holding Time on Concentrations of Microorganisms in Water Samples*, Water Environ Res., 76:67-72 (2004), attached as Exhibit 5; and Pope, et al., *Assessment of the Effects of Holding Time and Temperature on Escherichia coli Densities in Surface Water Samples*, Appl. Environ. Microbiol., 69:6201-07 (2003), attached as Exhibit 6.

⁵ Nineteen of the above samples only had the date of set-up recorded. For these samples, the average set-up time was used. *See* Ex. 3 (Olsen Aff., ¶ 14).

See Ex. 3 (Olsen Aff., ¶ 14).⁶ As shown, most samples were set-up for analysis within 30 hours.

The exception is for the high flow station samples⁷ and the edge of field samples.⁸

In sum, Dr. Harwood states:

⁶ Appendix A to Defendants' Motion purports to calculate holding times for selected samples. The "Hold Time (Analysis)" is shown to range from 3 to 13 days. This is not an accurate calculation; indeed, it is misleading. *See* Ex. 3 (Olsen Aff., ¶ 13). The hold time should be calculated from sample collection time to analysis set-up (or prep) time. *See id.* Analytical methods require that the bacteria be grown on cultures and the concentrations (densities) be recorded at set times up to 48 hours before the results are analyzed. *See id.* But that time period does not constitute the hold time.

⁷ During the first year of sampling in 2005, automatic samplers were installed at selected stream locations to collect storm runoff samples (*i.e.*, the high flow stations or "HFS"). *See* Ex. 3 (Olsen Aff., ¶ 7). These samplers collected water into bottles over approximately 30 to 36 hours to obtain samples over the total high flow event, from start to finish. *See id.* After determining the flow conditions throughout the collection process (as recorded by the automatic samplers), the samples from the bottles were then composited to create one flow-weighted sample that was sent to the laboratory for analyses. *See id.* This compositing was performed in the CDM laboratory in Denver. *See id.* Therefore, several days could occur between start of sample collection and arrival at the laboratory. *See id.* At the advice of Dr. Harwood, this practice was discontinued for the bacteria samples. *See id.* Instead grab samples were collected during the high flow event (typically at the highest flow) and sent to the laboratory on the same day of collection. *See id.* The initial practice resulted in some samples not being shipped to the laboratory on the same day of collection and thus longer hold times before the sample analysis. *See id.* Even with these initial longer times before laboratory set-up, the majority of the HFS samples analyzed for bacteria arrived at the laboratory and were set-up in less than 48 hours. *See id.*

⁸ Similar to high flow station samples collected with the automatic samplers, various other samples with high suspended solids and high organic content (*i.e.*, storm samples and edge of field samples) required extra time for filtering and, as a result, sometimes the field staff could not ship these samples the same day. *See* Ex. 3 (Olsen Aff., ¶ 8). In some cases, these samples were also shipped to the CDM Denver laboratory for processing because the Denver laboratory had larger and more processing equipment (vacuum pumps, filtration apparatus, etc.). *See id.* After review of this procedure, field staff were instructed to send samples for bacteria analyses directly to EML from the field on the same day of collection. *See id.* This was reflected in CDM SOP 10-1, Edge of Field Sampling, which provided that: "Samples for bacteria will be placed into a sterile bottle and shipped directly to the laboratory. The remaining sample will be processed (filtered and preserved) as appropriate at the CDM laboratory in Denver or the staging facility in Tulsa." *See id.* Because of shipping to the Denver laboratory for processing, some samples for bacteria analyses had longer times before they were set-up and analyzed at EML. *See id.* In particular some of the field runoff samples (edge of field ("EOF")) had longer times before set-up and analysis. *See id.*

My expert opinion, based upon the literature reports and work conducted in my laboratory, is that the data from the samples collected for the State by CDM are useful and scientifically valid for assessing the extent of microbial contamination in IRW waters. If anything, the data from samples held longer than 30 hours will tend to underestimate the microbial contamination in these waters, particularly for *E. coli* and fecal coliform concentrations. The enterococci concentrations should not change significantly even with a 48 hour holding time, and any changes that occur with longer hold times should be a decrease in concentration. The data collected for this study do not exaggerate microbial contamination and associated human health risks in the IRW. If anything, they are an underestimate due to the tendency of indicator bacteria to die off with increased holding times.

See Ex. 1 (Harwood Aff., ¶ 9).

II. Legal Standard.

The basis for admitting the testimony at issue such as that provided by Dr. Harwood, Dr.

Olsen and Dr. Teaf is Rule 702 of the Federal Rules of Evidence:

If scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of an opinion or otherwise, if (1) the testimony is based upon sufficient facts or data, (2) the testimony is the product of reliable principles and methods, and (3) the witness has applied the principles and methods reliably to the facts of the case.

As an initial matter, the court must determine if the expert is qualified by "knowledge, skill, experience, training, or education" to render an opinion. *See id.* In connection with this Motion, Defendants do not contest Dr. Harwood's, Dr. Olsen's and Dr. Teaf's expertise in the subject areas in which they will testify. Indeed, a review of their experience and qualifications indicates they are indeed experts in their respective fields. *See* Ex. 7 (Harwood Report, ¶¶ 1-3); Ex. 8 (Harwood CV); Ex. 1 (Harwood Aff., ¶¶ 1-2); Ex. 9 (Olsen CV); Ex. 2 (Olsen Aff., ¶¶ 1-2); Ex. 10 (Teaf Report, ¶¶ 2-9); Ex. 11 (Teaf CV).

Next, a court must ensure that the scientific testimony being offered is "not only relevant, but reliable." *See Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 509 U.S. 579, 589 (1993).

This is the issue raised by Defendants' challenge of the reliability of the analyses of the water samples collected by CDM and the opinions offered by the State's experts that rely upon those analyses. "To be reliable under *Daubert*, an expert's scientific testimony must be based on scientific knowledge" *Dodge v. Cotter Corp.*, 328 F.3d 1212, 1222 (10th Cir. 2003). The Supreme Court has explained that the term "scientific" "implies a grounding in the methods and procedures of science." *Daubert*, 509 U.S. at 590.

The Supreme Court has set forth four non-exclusive factors that a court may consider in making its reliability determination: (1) whether the theory or technique can be (and has been) tested, *id.* at 593; (2) whether the theory or technique has been subjected to peer review and publication, *id.*; (3) the known or potential rate of error and the existence and maintenance of standards controlling the technique's operation, *id.* at 594; and (4) whether the theory or technique has general acceptance in the scientific community, *id.* Importantly, the Supreme Court cautioned that the inquiry is "a flexible one." *Id.*; *see also id.* at 593 ("[m]any factors will bear on the inquiry, and we do not presume to set out a definitive checklist or test"); *Dodge*, 328 F.3d at 1222 ("the list is not exclusive").

The Supreme Court has stated that it is not the conclusion reached by the expert, but rather the methods used to arrive at the conclusion that are at issue: "The focus [of the inquiry] . . . must be solely on principles and methodologies, not on the conclusions that they generate." *Daubert*, 509 U.S. at 595. The Tenth Circuit has stated the same principle this way:

The plaintiff need not prove that the expert is undisputably correct or that the expert's theory is "generally accepted" in the scientific community. Instead, the plaintiff must show that the method employed by the expert in reaching the conclusion is scientifically sound and that the opinion is based on facts which sufficiently satisfy Rule 702's reliability requirements.

Mitchell v. Gencorp Inc., 165 F.3d 778, 781 (10th Cir. 1999); *see also Truck Ins. Exchange v.*

Magnietek, Inc., 360 F.3d 1206, 1210 (10th Cir. 2004).

Thus, a litigant must show only that the method used by an expert is scientifically sound and that the expert's opinion is based on sufficient facts to satisfy the reliability requirement of Rule 702. *See Mitchell*, 165 F.3d at 781; *see also In re Paoli R.R. Yard PCB Litig.*, 35 F.3d 717, 744-45 (3d Cir. 1994). The Third Circuit in *In re Paoli*, highlighting the "good grounds" requirement of *Daubert* noted that the reliability standard is lower than the merits standard of correctness. *Id.* Further, the Third Circuit noted that:

The grounds for the expert's opinion merely have to be good, they do not have to be perfect. The judge might think that there are good grounds for an expert's conclusion even if the judge thinks that there are better grounds for some alternative conclusion, and even if the judge thinks that a scientist's methodology has some flaws such that if they had been corrected the scientist would have reached a different result.

In re Paoli, 35 F.3d at 744. In the instant case, it is clear from the evidence provided that the analyses of the CDM samples were conducted pursuant to scientific methods that are both "generally accepted" and based on "good grounds."

III. Argument

A. The EPA, USGS and ODEQ hold-time standards used for regulatory and compliance purposes are not applicable; the analyses of the CDM samples are reliable

Defendants' assertion that the hold times for the CDM samples were required to comport with the EPA, USGS and ODEQ standards for regulatory and compliance purposes is incorrect. The samples here were not taken for either of those purposes. Rather, the purpose of analyzing the IRW water samples was to add to existing data collected by the State of Oklahoma and the USGS regarding the extent of bacterial contamination in the waters of the IRW and the percentage of samples that exceeded State and federal water quality guidelines. *See Ex. 1* (Harwood Aff., ¶ 4). Therefore, the EPA, USGS and ODEQ standards -- standards that

Defendants base their entire argument on -- are simply not controlling. In fact, generally accepted practice in the non-regulatory setting (such as here) allows for significantly longer hold times. *See, e.g.*, Ex. 1 (Harwood Aff., ¶ 7) (noting, for example, that the American Public Health Association's *Standard Methods for the Examination of Water and Wastewater* (2005) stipulates that ambient water samples collected for non-regulatory purposes can be held for 24 hours at cold temperatures before analysis).⁹ Thus, contrary to Defendants' bald assertion, *see* Motion, p. 4, the use of longer hold times is not a "new theory."

Indeed, in addition to the American Public Health Association's *Standard Methods for the Examination of Water and Wastewater*, the literature is clear that hold times significantly longer than those set out in the EPA, USGS and ODEQ standards yield scientifically valid results. *See* Ex. 1 (Harwood Aff. ¶¶ 6-9) (citing published studies that reflect that samples hold times of up to 48 hours resulted in no change, or a decrease in bacterial concentrations). The effect, if any, of an extended hold time is that bacterial concentrations will be *lower* than if the analysis was done immediately. *See* Ex. 1 (Harwood Aff. ¶ 6). The purpose for which these sampling analyses would be offered to the jury is to show the high levels of bacteria in the waters of the IRW. That these sampling analyses might be conservative in their reflection of the bacterial levels -- something that would inure to the benefit of Defendants -- in no way makes them unreliable for the purposes they would be offered. In fact, as Dr. Harwood states, "[m]y expert

⁹ Even Defendants' expert Dr. Samuel Myoda does not embrace Defendants' extreme position that the EPA, USGS and ODEQ regulatory and compliance standard is the mandatory standard. Dr. Myoda writes in his report, p. 10: "It is imperative that standard methods that have been accepted by the scientific community are followed. These methods should be approved by the appropriate authority such as the EPA, *Standard Methods for the Examination of Water and Wastewater*, and / or AOAC, etc." (Emphasis added.) The Standard Methods for the Examination of Water and Wastewater, as noted above, provides for hold times of up to 24 hours -- four times longer than the hold times Defendants seek to impose as the *sine qua non* of reliability.

opinion, based upon the literature reports and work conducted in my laboratory, is that the data from the samples collected for the State by CDM are useful and scientifically valid for assessing the extent of microbial contamination in IRW waters." *See* Ex. 1 (Harwood Aff., ¶ 9).

In sum, as demonstrated by the facts set out above, there has been extensive research regarding hold times greater than six hours. They can be tested, have been subjected to peer review and publication, and have a known or potential rate of error. Moreover, hold times greater than six hours for sampling done for purposes other than regulatory compliance have general acceptance in the scientific community. *See, e.g.,* Ex. 1 (Harwood Aff., ¶¶ 6-9) (citing studies and experiences in own laboratory). As such, the analyses of the CDM samples that have hold times greater than six hours are grounded in science and are reliable under *Daubert*. *See* 509 U.S. at 593.

B. The CDM samples were handled properly

Defendants assert that there was a so-called "96 Hour Hold Time" procedure put in place by CDM at the behest of Dr. Olsen. *See* Motion, pp. 10-14. This is incorrect. As the facts above make clear, the procedures put in place provided that samples would be shipped overnight to EML the same day as the samples were collected, *see* Ex. 2 (Olsen Aff., ¶¶ 5-6 & 9). Moreover, not only were they shipped in a manner to ensure the proper temperature, *see* Ex. 2 (Olsen Aff., ¶ 5), but also they arrived at EML at the proper temperature. *See* Ex. 3 (Sambasivam Aff., ¶ 3). Thus, contrary to Defendants' assertion, *see* Motion, p. 2, the samples were not "useless" when they reached EML.

C. Defendants grossly exaggerate the hold times of the CDM samples

With their Appendix A, Defendants attempt to assert that the CDM samples endured lengthy hold times. Defendants' assertion is flawed because it reflects a fundamental

misunderstanding of hold times. Hold times should be calculated from sample collection time to analysis set-up (or prep) time, *see* Ex. 1 (Olsen Aff., ¶ 9), and should not include the time during which the bacteria is being cultured before the results are analyzed. *See id.* EML, it should be recalled, *always* started and performed the set-up for the bacterial analyses (the cultures) on the day the CDM samples were received. *See* Ex. 3 (Sambasivam Aff., ¶ 3). An accurate reflection of the hold times for the various types of CDM samples is reflected in the table on page 6, above. Plainly, analyses of the vast majority of the samples that are the subject of Defendants' Motion began within 24 to 30 hours from sample collection. *See* Ex. 1 (Harwood Aff., ¶ 4.)

* * *

In sum, the hold times used for analyzing the CDM samples are a generally accepted method in the field of bacterial sampling analyses. Moreover, the CDM samples were properly handled during the hold times. Therefore, the analyses of these samples were reliably conducted and have resulted in reliable, scientifically valid data. Expert opinions based upon these samples are thus admissible under *Daubert*.

IV. Conclusion

All of the *Daubert* factors weigh in support of reliability of the sampling analyses. The research demonstrating the scientific acceptability of the hold times occurring was not litigation-driven and was not developed by counsel. Accordingly, Defendants' Motion should be denied in all respects.

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